



Mineral fertilizer alters cellulolytic community structure and suppresses soil cellobiohydrolase activity in a long-term fertilization experiment

Fenliang Fan^{a,1}, Zhaojun Li^{a,1}, Steven A. Wakelin^b, Wantai Yu^c, Yongchao Liang^{a,*}

^aKey Laboratory of Plant Nutrition and Fertilization, Ministry of Agriculture, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, South Zhongguancun Street No. 12, Beijing 100081, China

^bAgResearch Ltd, Lincoln Science Centre, Christchurch 8140, New Zealand

^cLaboratory of Nutrients Recycling, Institute of Applied Ecology, Chinese Academy of Sciences, 72 Wenhua Road, Shenyang 110016, China

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ABSTRACT

Nutrient inputs to soil can alter mineralization of organic matter and subsequently affect soil carbon levels. To understand how elemental interactions affect the biogeochemistry and storage of soil C, we examined soils receiving long-term applications of mineral fertilizer and manure-containing fertilizers. As cellulose is the dominant form of carbon entering arable soils, cellulolytic communities were monitored through enzymatic analysis, and characterization of the abundance (real-time PCR) and diversity (terminal restriction fragment length polymorphism, T-RFLP) of fungal cellobiohydrolases (*cbh1*) genes. The data showed that long-term mineral fertilization increased soil organic C and crop productivity, and reduced soil heterotrophic respiration and cellobiohydrolases (CBH) activity. Correspondingly, the diversity and community structure of cellulolytic fungi were substantially altered. The variation in cellulolytic fungi is mainly attributable to shifts in the proportion of Eurotiomycetes. In addition, CBH activity was significantly correlated with the diversity and community structure of cellulolytic fungi. These results suggest that enhanced C storage by mineral fertilizer addition occurs not only from extra organic carbon input, but may also be affected through the cellulose decomposing community in arable soil.

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1. Introduction

Biogeochemical cycling in soil ecosystems is directly impacted following addition of exogenous nutrients (Edmeades, 2003; Knorr et al., 2005). Not surprisingly, the most affected soils are under agricultural land use where addition of agricultural fertilizer can significantly affect soil organic matter cycling (Parton et al., 1992; Edmeades, 2003; Zhang et al., 2009). This is a critical issue, as soil C storage is related to both soil structure and fertility, and also a sink or source of the greenhouse gas CO₂ (Edmeades, 2003; Lal, 2004). The direction and extent of shift in soil C cycling following fertilization can occur via two fundamental ways. Firstly, fertilization increases plant net primary productivity thus increasing overall inputs of organic C into soil (Edmeades, 2003; Jarecki and Lal, 2003; Yu et al., 2009). Secondly, fertilization changes the rate of microbial decomposition (heterotrophic mineralization of C) which controls carbon loss from soil as CO₂ (Carreiro et al., 2000; Waldrop et al., 2004; Ding et al., 2007). Since long-term inputs of organic C to

soil do not necessarily culminate in increased total C (Gill et al., 2002; Khan et al., 2007; Zhang et al., 2009), C accumulation may be more tightly coupled to microbial decomposing processes than to organic C inputs *per se*.

Knowledge of how nutrient inputs influence the microbial cycling of organic C in natural ecosystems, especially in regards to key taxa involved in this function, is rapidly accumulating. The analysis of soil extracellular enzymes associated with cycling of soil organic C, such as phenol oxidase and cellobiohydrolase, has revealed that these groups respond in a consistent manner to low level N addition in forests (Carreiro et al., 2000; Waldrop et al., 2004). In soils receiving litter of high lignin content, the residing microbial communities were dominated by Basidiomycete (white-rot) fungi. N increased C storage in these soils by down-regulating expression of ligninolytic *lcc* gene and reducing laccase activity (Carreiro et al., 2000; Waldrop et al., 2004; Blackwood et al., 2007; Edwards et al., 2011). In soil systems in which lignin content is low and other fungi are dominant (e.g. Ascomycetes), low levels of N may increase decomposition (Blackwood et al., 2007). Furthermore, the association between phenol oxidase activity and laccase gene abundance and diversity has been shown to co-vary temporally (Blackwood et al., 2007; Artz et al., 2009), indicating that associated effects on C-cycling in some

* Corresponding author. Tel.: +86 10 8210 8657; fax: +86 10 8210 6225.

E-mail address: ycliang@caas.net.cn (Y. Liang).

¹ These authors contributed equally to this work.